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The purpose of our project is to investigate the effects of soy isoflavone supplementation on biomarkers of growth and differentiation on the breast tissues of women with breast cancer scheduled for mastectomy or lumpectomy. Seventy-two women will be randomly assigned to receive placebo or soy isoflavone tablets for three weeks prior to surgery. Blood samples before and after supplementation will be collected to measure serum isoflavones and other micronutrients. Tissue samples from benign and malignant areas of the surgical specimens will be analyzed by Western blotting, immunohistochemistry and histopathology in both groups to determine the effect of supplementation on biomarkers of growth (MIB-1, EGFR, cyclin D1, CDK5, CDK6), differentiation (Cx43, E-cadherin) and apoptosis (bcl-2, bax, p21, p53, Rb).

During the first year of the project, we have 6 patients entered on the study. There was a delay in starting the project due to difficulty hiring of study personnel and changes of personnel. There was also a change made in the study protocol requiring approval by IRB. Originally we had proposed using soy protein isolate. However, we switched from soy protein isolate to isoflavone tablets, because of better compliance expected with tablets. Currently no data are available for reporting.

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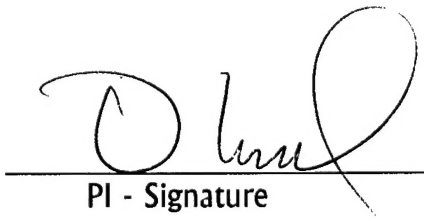

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INTRODUCTION

Epidemiological studies have shown an inverse association between dietary intake of fruits and vegetables and carcinoma of the breast. One group of major micronutrients in vegetables and fruits which have been postulated to prevent breast cancer are soy isoflavones. The mechanism by which isoflavones may prevent breast cancer is not known. Based on our preliminary studies, we hypothesize that isoflavones inhibit cell proliferation, upregulate the expression of gap junctional protein connexin 43 (cx43) and alter the expression of cell cycle regulatory proteins. Our studies will investigate the *in vivo* effects of isoflavones on human breast tissues obtained from lumpectomy/mastectomy specimens. We will investigate the effect of increased tissue concentration of isoflavones for a period of three weeks on breast cell proliferation, differentiation and cell cycle regulatory proteins. Sixty-four patients with ductal carcinoma *in situ* (DCIS) or invasive breast cancer scheduled to have surgery will be randomly assigned to supplement their diet with 100 mg soy isoflavone or placebo daily for three weeks. Plasma isoflavone levels will be measured at baseline and after three weeks in both groups. Tissue isoflavone levels will be measured on samples from surgical specimens in benign and malignant areas of the epithelia in both groups. Biomarker studies will be done on surgical specimens by immunohistochemistry and Western blot analysis. Comparisons will be made between areas of comparable microscopic characteristics [malignant, DCIS, lobular carcinoma *in situ* (LCIS), dysplasia, hyperplasia and benign] on breast tissues of patients from intervention and control groups. These studies will enable us to determine if a short duration of exposure to increased tissue levels of isoflavones will modulate biomarkers of cell differentiation (cx43), adhesion (E-cadherin), proliferation (MIB-1), and cell growth and apoptosis (bcl-2, bax, p53, p21,Rb, EGF-R, cyclin D1, CDK5, CDK6) in benign, pre-malignant and malignant areas of breast epithelial tissues. In addition, baseline biopsy samples are available in all patients, and a limited number of the marker studies (prioritized in the order cx43, bcl-2, p21, CDK5) will be performed on pre-intervention biopsy samples of patients in the intervention group, giving us an opportunity to compare pre- and post-intervention marker levels in the same

BODY

During the first year of the study, 6 subjects were enrolled. The low accrual rate was due to the delay in getting the study started because of difficulty in hiring study personnel and change of study personnel. An additional delay in starting the study was due to changes made in the study design by introducing isoflavone and placebo tablets and making patients with invasive cancer eligible. The study intervention was changed from soy protein isolate to soy isoflavone tablet, in order to make the study intervention easier to take and to improve the compliance with the study intervention. The change also improved the study design by introducing a placebo arm instead of a no intervention arm. The study design is now better both scientifically and practically, because it is easier for the patients to accept a placebo controlled study compared to one with no intervention arm. However, these necessary changes resulted in additional delays in starting the study because of resubmissions to the IRB.

The study is currently accruing at a rate of two patients per month, which is sufficient to complete the study on time in accordance with the objectives stated in the grant application. In our proposal the predicted accrual rate was 1.6 subjects per month.

No data are available at the time of this report, as tissue samples from at least 6 more subjects will be collected before performing the tissue marker studies. The blood and tissue samples will be analyzed in batches of 10-20 to increase efficiency and to keep the study costs within budgetary guidelines.